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# REPORT ON RESEARCH IN EGYPT TO SELECT PAPAVER SOMNIFERUM PLANTS WITH A HIGH MORPHINE CONTENT

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1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

2/ Professor G.H. Mahran and Dr. I. Salad Ahmed, Faculty of Pharmacy, Cairo University, collaborated in this research project.

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

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## INTRODUCTION

Progress in medical and related health services throughout the world has increased the demand for codeine, a widely used antitussive and mild analgesic. To meet this expanding demand, adequate supplies of this drug must be ensured. Therefore, new technology is required to increase the yield per unit area of phenanthrene alkaloids.

The purpose of this research is (1) to identify Papaver somniferum plants with a high morphine content which could be used in other countries to develop superior varieties and (2) to determine the number of successive seed crops that can be grown in Egypt in a 12 months period.

## METHOD AND MATERIALS

Experiments were conducted in collaboration with the Faculties of Pharmacy at the Universities of Alexandria, Asyût and Cairo, representing a north to south distance of 570 km. Alexandria has a Mediterranean climate while Cairo and Asyût have desert climates.

Papaver somniferum seed from the following six sources was provided by the U.N. Narcotics Laboratory and it was used in the experiments at each location:

| | | | | |
|---|---|---|---|---|
| UNS 1 | P. somniferum | seed | from | Hungary |
| UNS 2 | " | " | " | Norway |
| UNS 3 | " | " | " | Switzerland |
| UNS 4 | " | " | " | Hungary |
| UNS 13 | " | " | " | Spain |
| UNS 39 | " | " | " | Turkey. |

At Alexandria the soil was a loam; at Cairo and Asyût a clay/loam. Chemical and mechanical soil analyses for the experimental site at Cairo are given in Table 1. At the three locations, the experiments consisted of six square plots. Each plot was divided into six lines (planting beds) 2.5 m in length and 70 cm between lines. Two rows spaced 20 cm apart were sown on each

line. The seeds were mixed with sand and an aliquot with 8 to 10 seeds was sown in shallow pits 10 cm in length and covered with a thin layer of soil. The soil was irrigated to maintain the required level of moisture for germination and plant growth.

The dates of sowing the experiments were:

| | |
|---|---|
| Alexandria | 17/10/75 |
| " | 12/5/76 |
| Cairo | 9/4/75 |
| " | 10/11/75 */ |
| " | 26/1/76 |
| " | 17/3/76 |
| " | 25/5/76 |
| Asyût | 20/1/76 |
| " | 15/3/76 |
| " | 20/5/76 |

*/ Initial sowing made on 14/10/75; resown due to poor seedling emergence.

After seedling emergence the plants were thinned to two per pit, spaced approximately 50 cm apart within the row. At the time of thinning, excess vigorous seedlings were transplanted to other plots arranged in the same way as those for the initial sowings.

After the young plants were making good growth, all plots were fertilized with $(NH_4)_2SO_4$ and $K_2SO_4$ at the rate of 50 kg of each per feddan (4,200 $m^2$). Weeds were controlled by hand cultivation.

At maturity, the capsules from each plant were harvested when pale straw to yellowish green in colour, placed in a separate container, assigned an identification number and air-dried. Later, the capsules were cut transversely; the seed collected and given the plant capsule identification number. The weight of the capsules without seed was recorded for each plant.

The following thin-layer chromatography procedure was used to quantify the morphine content of harvested capsules :

1. One gramme, weighed accurately, of each sample was introduced in an extraction flask, macerated overnight with 6 ml of solvent mixture (chloroform:isopropanol:ethanol:ammonium hydroxide in ratios 6:2:1.5:0.5 by volume). Flasks were shaken and extracts were filtered in 10 ml measuring flasks. Extraction was completed and volume adjusted to 10 ml.
2. Extracts were spotted on plates of silica gel 0.25 mm thickness along with spots of standard morphine solutions corresponding to 3,5 and 7 µg morphine.
3. Plates were developed with a solvent system acetone:benzene:methanol: ammonium hydroxide in ratios 99:83:10:8 by volume.
4. The developed chromato-plates were sprayed with 1% aqueous picric acid, dried at 60-70°C then sprayed with 2% aqueous iodine.
5. Plates were examined under U.V. (254 mµ).

The weighted average percent morphine content for each UNS number was calculated from the weight of capsules and morphine yield per plant.

## RESULTS AND DISCUSSION

### Cairo

Seed of the six P. somniferum selections sown on 10 November 1975 gave useful observations on time of sowing, plant growth and development and morphine content in the capsules of individual plants. A summary of observations on several plant characteristics under study is given in Table 2.

The number of plants in each seed source ranged from 93 to 143. Generally, the plants grew satisfactorily and many reached a maximum height at maturity in the range of 100 to 155 cm except UNS-13 which was only 35 cm. Scorching symptoms on the leaves was generally evident among the plants; UNS-1 and UNS-4 showed the least damage. All plants were attacked by a fungus at the time of flowering which hindered capsule development.

The best plant growth and flower development occurred in UNS-4 followed by UNS-1 and UNS-39. Although growth was slower in UNS-2 and UNS-3, it was by comparison much poorer in UNS-13. Either the latter ecotype is morphologically different from the others or the local environmental pressures restricted plant growth, flowering and capsule size.

UNS-4 was the earliest flowering seed source. The first flower was noted on 26 February. UNS-13 was latest to flower, producing the first flower on 5 April.

Of significant importance was the variation among seed sources in the proportion of plants producing mature capsules. In UNS-1 and UNS-4 79% and 69% respectively of the plants produced usable capsules. The proportion of capsule bearing plants for the other seed sources was much lower: 38% for UNS-3; 37% for UNS-39; 31% for UNS-13 and 30% for UNS-2. Thus the total yield of capsules per unit area was significantly reduced for the latter four seed sources.

The average number of capsules per plant ranged from 5.8 for UNS-39 to 2.4 for UNS-2. One plant in the UNS-39 population produced 42 capsules. The average weight of capsules per plant varied from 5.1 g for UNS-4 to 0.8 g for UNS-13. The latter had very small capsules measuring 1.5 cm in length by 1 cm in width.

The morphine content for the different seed sources was 0.58% in UNS-13; 0.55% in UNS-39; 0.46% in UNS-2; 0.44% in UNS-4 and 0.39% in UNS-1. The percentages of morphine fall generally within the normal range for straw. However, the morphine content of UNS-13 is much lower than that found in capsules grown in Spain.

In identifying plants for further investigation, the quantity and concentration of morphine were the primary selection criteria. Table 3 shows the distribution of plants within each UNS seed source according to the quantity of morphine they produced. Plants in the populations of UNS-1, UNS-4 and UNS-39 yielded more than 6.99 mg of morphine. UNS-4 had the most plants in the higher yield classes. After one or two additional cycles of evaluation,

seed of plants producing the largest quantity of morphine will be forwarded to countries breeding new varieties of P. somniferum with higher yield of phenanthrene alkaloids per unit area.

Table 5 also gives the distribution of plants within each UNS number according to the percent of morphine. Plants with a morphine content of 0.7% or higher were found in each of the UNS seed sources. Of the 344 individual plants analysed by the TLC method in this experiment, 60 or 17% had capsules with a morphine concentration of 0.7% or better. The high morphine plant selections will be included in the next evaluation test at Cairo. In addition seed from a number of these plants will be sown at Asyût to obtain information on the effect of environment on morphine content.

Experiments sown at Cairo on 9 April 1975, 26 January 1976, 17 March 1976 and 25 May 1976 failed to produce usable plant materials. Seeds sown on 9 April germinated and produced seedling plants. However, the high temperatures which prevail at that time of year together with the Khamasin winds caused most of the plants to wither and die. Climatic conditions were unfavourable for the sowing made on 26 January. No plants grew to the flowering stage. Seed germination was poor following the 17 March and 25 May sowings. Temperatures were too high and the seedling plants which did emerge were weak and soon died. Thus results from the date of sowing research show the best time to sow P. somniferum seed is from early October to mid-November. This period will provide the maximum germination, seedling emergence and plant growth. Sowings made at other dates encounter unfavourable weather conditions for germination and the plants which emerge wither and die or fail to make normal growth, flower or produce mature capsules.

Generally, seedlings transplanted at the time of thinning did not survive. Only a few trans-plants of UNS-5 and UNS-39 produced mature capsules. They had a lower average morphine content probably caused by the delay in growth due to the "shock" of trans-planting.

Alexandria

Neither of the experimental sowings were successful at this location because of unfavourable climatic conditions. It can be concluded that the

propagation of P. somniferum germplasm is not practical under the specific conditions at this site.

Asyût

Sowings of P. somniferum seed on 20 January 1976, 15 March 1976 and 25 May 1976 were not successful. Although seed germination and plant growth was satisfactory following the earlier sowing, burning symptoms and fungus attacks on the leaves hindered flowering and capsule maturation. In the entire experiment, only six plants of UNS-4 and two plants of UNS-39 had usable capsules.

Unfavourable climatic conditions, particularly increasing temperatures, following the sowings on 15 March and 25 May caused the seedling plants to wither and die. In the March sowing 3 plants of UNS-4 and two plants of UNS-39 persisted for about one month then faded and died also. In the sowing made in May none of the seed germinated due to high soil temperatures.

New experiments will be sown in late October 1976.

## CONCLUSIONS

1. Sixty individual plants of P. somniferum in the first cycle of evaluation were identified having capsules with a morphine content of 0.7% or greater as determined by HLC analyses.

2. a) At Cairo, UNS-4 and UNS-39 gave the largest percentages of high morphine yielding plants (mg morphine per plant);
   b) UNS-4 and UNS-39 had the largest number and weight of capsules per plant;
   c) There were significant differences among the six UNS seed sources in percentage of plants producing mature capsules;
   d) UNS-13 followed by UNS-39 had the highest average morphine percentage;
   e) UNS-39 had the largest percentage of plants with a morphine content of 0.7% or greater.

3. Fall sowing (mid-October to mid-November) of poppy seed at Cairo provides the best climatic conditions for germination, seedling emergence and normal plant growth and development. Sowings in January, March, April and May have been failures due to unfavourable climate and diseases.

4. Seed sown at Alexandria in October and May, and at Asyût in January, March and May failed to develop into usable plant materials.

5. The research site near Alexandria is unsuitable for the experiments with the opium poppy.

6. Transplanting seedlings from fall sowings at Cairo resulted in a high plant mortality, reduced growth and a lower morphine content.

7. Seed from each of the 60 selected plants will be sown in a second cycle evaluation test in October 1976.

Table 1

Mechanical and chemical analyses of the soil in the experimental field at Cairo, Egypt

Mechanical analysis:

| | |
|---|---|
| Coarse sand | 4.10% |
| Fine sand | 34.22% |
| Silt | 27.70% |
| Clay | 22.85% |
| $Ca\ CO_3$ | 3.07% |
| Texture | Clay loam |

Chemical analysis:

| | |
|---|---|
| Total soluble salts | 0.35% |
| $H\ CO_3$ | 1.20% |
| Cl | 1.56% |
| $SO_4$ | 1.70% |
| Ca | 1.60% |
| Mg | 0.85% |
| Na | 2.50% |
| Available N | 29.20 ppm |
| Available P | 20.70 ppm |
| Available K | 755.00 ppm |
| pH | 7.9 |

Table 2

Observations on Papaver somniferum plants from different seed sources at Cairo University 1/

| | Seed sources from United Nations Laboratory | | | | | |
|---|---|---|---|---|---|---|
| | UNS-1 | UNS-2 | UNS-3 | UNS-4 | UNS-13 | UNS-39 |
| PLANTS | | | | | | |
| Date of emergence | 23/11/75 | 25/11/75 | 25/11/75 | 22/11/75 | 23/11/75 | 24/11/75 |
| No. after thinning | 101 | 120 | 101 | 143 | 98 | 93 |
| Height at maturity (cm) | 120 - 155 | 65 - 130 | 60 - 100 | 90 - 145 | 30 - 35 | 80 - 110 |
| Stem diameter (cm) | 5 | 5 | 4 | 4 | 2 | 6 |
| No. with mature capsules | 80 | 36 | 38 | 99 | 30 | 34 |
| LEAVES | | | | | | |
| Length (cm) | 40 - 55 | 50 - 58 | 50 - 58 | 40 - 60 | 30 - 37 | 40 - 55 |
| Breadth (cm) | 18 - 21 | 17 - 22 | 22 - 28 | 18 - 25 | 16 - 20 | 14 - 22 |
| FLOWERS | | | | | | |
| Date of first flowering | 10/3/76 | 25/3/76 | 28/3/76 | 26/2/76 | 5/4/76 | 1/3/76 |
| No. of flowering plants | | | | | | |
| at 3/4 | 45 | 3 | 2 | 55 | -- | 40 |
| at 6/4 | 56 | 11 | 5 | 65 | 2 | 60 |
| at 10/4 | 60 | 20 | 15 | 70 | 12 | 80 |
| at 12/4 | 70 | 40 | 25 | 90 | 25 | 80 |
| Colour of petals | White/Crimson base | White/pale rose base | White/Crimson base | White/Pale violet base | White/red base | Deep/Violet to Crimson base |
| No. of stigmas/flower | 13 - 14 | 12 - 15 | 12 - 14 | 13 - 14 | 12 - 14 | 11-- 12 |
| CAPSULES | | | | | | |
| No. at 3/4 | 61 | 4 | -- | 189 | -- | 47 |
| No. at 12/4 | 290 | 62 | 69 | 417 | 20 | 194 |
| Total No. | 327 | 86 | 129 | 485 | 109 | 199 |
| Size: Length (cm) | 5 | 3 | 3 | 5 | 1.5 | 4 |
| Breadth (cm) | 3 | 3 | 3 | 4 | 1.0 | 4 |
| Average number per plant | 4.1 | 2.4 | 3.4 | 4.9 | 3.6 | 5.8 |
| Average wt/plant | 4.1 | 1.6 | 1.6 | 5.1 | 0.8 | 4.5 |
| Morphine content % 2/ | 0.39 | 0.46 | 0.43 | 0.44 | 0.58 | 0.55 |

1/ Date of sowing: 10.11.75; Date of thinning: 26.12.75; Date of harvest: 18.4.76.

2/ Weighted average morphine content of individual plant capsules in each UNS seed source.

Table 3

Frequency distributions of the quantity and concentration of morphine in P. somniferum produced from different seed sources at Cairo University

| | Seed sources from United Nations Laboratory | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UNS-1 | | UNS-2 | | UNS-3 | | UNS-4 | | UNS-13 | | UNS-39 | |
| No. of plants | 80 | | 34 | | 38 | | 99 | | 30 | | 34 | |
| Quantity of morphine | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Less than 1 mg | 2 | 2.50 | 2 | 5.88 | 2 | 5.26 | - | - | 11 | 36.66 | - | - |
| 1-1.99 mg | 9 | 11.25 | 10 | 29.41 | 22 | 57.89 | 5 | 5.05 | 14 | 46.66 | 2 | 5.88 |
| 2-2.99 mg | 19 | 23.75 | 4 | 11.76 | 8 | 21.05 | 18 | 18.18 | 4 | 13.33 | 8 | 23.52 |
| 3-3.99 mg | 19 | 23.75 | 7 | 20.58 | 4 | 10.52 | 21 | 21.21 | - | - | 6 | 17.64 |
| 4-4.99 mg | 13 | 16.25 | 7 | 20.58 | 1 | 2.63 | 20 | 20.20 | 1 | 3.33 | 4 | 11.76 |
| 5-5.99 mg | 6 | 7.50 | 3 | 8.82 | 1 | 2.63 | 14 | 14.14 | - | - | 7 | 20.58 |
| 6-6.99 mg | 7 | 8.75 | 1 | 2.94 | - | - | 8 | 8.08 | - | - | 3 | 8.82 |
| 7-7.99 mg | 3 | 3.75 | - | - | - | - | 7 | 7.07 | - | - | 1 | 2.94 |
| 8-8.99 mg | - | - | - | - | - | - | 5 | 3.03 | - | - | 2 | 5.88 |
| 9-9.99 mg | 1 | 1.25 | - | - | - | - | 2 | 2.02 | - | - | 1 | 2.94 |
| More than 10 mg | 1 | 1.25 | - | - | - | - | 1 | 1.01 | - | - | - | - |
| Total % | | 100 % | | 99.97 | | 99.98 | | 99.99 | | 99.98 | | 99.96 |
| Concentration of morphine | | | | | | | | | | | | |
| Less than 0.3 % | 14 | 17.50 | 1 | 2.94 | - | - | 3 | 3.03 | - | - | - | - |
| 0.3 % | 35 | 43.75 | 14 | 41.17 | 21 | 55.26 | 38 | 38.38 | - | - | 3 | 8.82 |
| 0.4 % | 1 | 1.25 | - | - | - | - | - | - | - | - | - | - |
| 0.5 % | 21 | 26.25 | 12 | 35.29 | 12 | 31.58 | 39 | 39.39 | 22 | 73.33 | 19 | 55.88 |
| 0.6 % | 1 | 1.25 | - | - | - | - | - | - | - | - | - | - |
| 0.7 % | 8 | 10.0 | 4 | 11.76 | 5 | 13.15 | 17 | 17.17 | 3 | 10.0 | 11 | 32.00 |
| More than 0.7 % | - | - | 3 | 8.82 | - | - | 2 | 2.02 | 5 | 16.66 | 1 | 2.94 |